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Minireview

Actin in the endocytic pathway: From yeast to mammals

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Abstract Genetic analysis of endocytosis in yeast early pointed to the essential role of actin in the uptake step. Efforts to identify the machinery involved demonstrated the important contribution of Arp2/3 and the myosins-I. Analysis of the process using live-cell fluorescence microscopy and electron microscopy have recently contributed to refine molecular models explaining clathrin and actin-dependent endocytic uptake. Increasing evidence now also indicates that actin plays important roles in post-internalization events along the endocytic pathway in yeast, including transport of vesicles, motility of endosomes and vacuole fusion. This review describes the present knowledge state on the roles of actin in endocytosis in yeast and points to similarities and differences with analogous processes in mammals.

Keywords: Actin; Myosin; Arp2/3; Formin; Endocytosis; Yeast

1. Actin in endocytic uptake

Genetic analysis of endocytosis in *Saccharomyces cerevisiae* early pointed to the essential role of actin in the uptake step. Not only mutations on actin cause internalization defects stronger than those observed in clathrin mutants [1,2] but also, a number of proteins directly involved in the control of actin dynamics are essential in the process (for review see [3,4]). A key player initiating actin polymerization at sites of endocytosis is the Arp2/3 complex [5,6]. Upon activation by nucleation promoting factors (NPFs), Arp2/3 induces actin polymerization and participates in assembly of branched actin networks that usually grow by adding monomers close to the surface where the NPF is located, typically a cellular membrane [7]. Addition of actin monomers pushes the NPF covered surface forward, either causing membrane deformation or rocketing of the membrane enclosed organelle [7]. Multiple NPFs participate in endocytic uptake in yeast, indicating that actin polymerization might be ignited at different points during membrane budding. The endocytic NPFs include the yeast WASP (Wiskott–Aldrich Syndrome Protein) homologue Las17 [8,9], the eps15 family member Pan1 [10,11], the actin binding protein Abp1 [12,13] and the unconventional type I myosins Myo3 and Myo5 [14,15]. Besides the force generated by local polymerization of actin, the mechanochemical activity of the type I myosin [15] might also actively contribute to deform the lipid bilayer. In response to ATP hydrolysis, the Myo5 N-terminal motor head is capable of translocating actin filaments with respect to its tail [15], which bears a phospholipid binding domain followed by the C-terminal extension that interacts with the actin polymerization machinery [16–19].

Development of live-cell imaging to study endocytic uptake very much contributed to order the sequence of molecular events occurring during endocytic budding and the role of the different NPFs in the process [15,20–26]. Upon clathrin arrival to the PM, other components of the endocytic coat including clathrin adaptors and Pan1 are assembled concomitant with recruitment of Las17 [20,21,23]. After a roughly 30 s period of restrained motility, the coat undergoes a 200 nm slow inward movement, which takes 10–15 s. Recruitment of the yeast amphiphysins Rvs161 and Rvs167 then precedes the fast movement of the coat into the cytosol [20]. Myo5 arrives to the endocytic patch at the onset of the slow inward movement, slightly after arrival of Arp2/3 and coincident with the start of massive actin polymerization [15,26]. Similar to Las17 and the amphiphysins, Myo5 is left at the PM as the vesicle moves into the cytosol [15,20,21,26]. The recruitment of Abp1 and a number of actin binding proteins such as fimbrin, cofilin and capping protein follows in an actin-dependent manner. Together with Arp2/3, these components remain associated with the vesicles as they move into the cytosol [20,21,25].

Based on the amphiphysin recruitment time point, it was proposed that the slow 200 nm coat inward movement corresponds to the initial PM invagination [27]. However, the resolution of the fluorescence microscopy techniques was insufficient to define when fission actually occurs. Idrissi et al. have recently demonstrated, using electron microscopy (EM), that endocytic internalization in yeast associates with the formation of tubular invaginations of about 50 nm in diameter and up to 180 nm in length, which accumulate the endocytic coat components at the tip [28]. Amphiphysin accumulates at the neck of the longest profiles confirming that fission occurs once the tubular profile has elongated (Fig. 1). Statistical analysis of the distribution of immuno-gold particles labeling different proteins along PM-associated tubular profiles of increasing length [28] has allowed refinement of models explaining the molecular forces driving membrane invagination [27] (Fig. 1, stage 3). Treatment of cells with the actin monomer sequestering drug Latrunculin A prevents the slow 200 nm coat inward movement [21], indicating that actin polymerization is essential to drive elongation of the incipient

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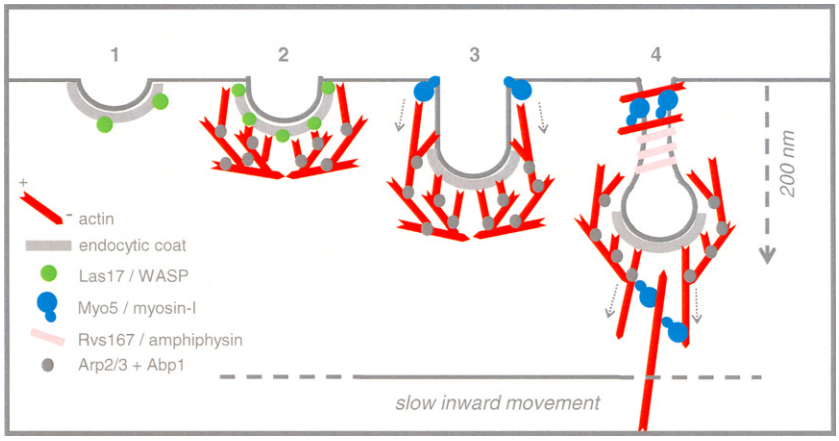

Fig. 1. Molecular model for clathrin, actin and type I myosin-dependent endocytic uptake in yeast. Based on live-cell fluorescence microscopy data (reviewed in [27]) and ultrastructural analysis of primary endocytic profiles [28,82] endocytic uptake in yeast might be dissected into the following steps: (1) assembly of an hemispherical clathrin coat previous to massive actin polymerization, (2) Arp2/3-dependent formation of an actin network on the surface of the endocytic coat induced by Las17 and Pan1, (3) elongation of the incipient invagination powered by Myo5-induced actin polymerization at the base of the profile and by the mechanochemical activity mediated by the myosin, (4) formation of two acto-myosin structures that cooperate with the yeast amphiphysins in the fission event by generating tension along the endocytic profile.

membrane invagination. FRAP (fluorescence recovery after photobleaching) experiments suggest that actin monomers are added close to the basal PM at this stage [20]. Consistently, the immuno-EM demonstrates that the NPF activity, mainly provided at this point by Myo5 [15], tightly localizes at the invagination base [28] (Fig. 1, stage 3). In addition to actin polymerization, the mechanochemical activity of the type I myosins contributes to power membrane invagination since mutation of the Myo5 ATPase in a *myo3*Δ background prevents endocytic uptake [29] and progression of the slow coat 200 nm inward movement [15]. Given the proposed polarity of the actin filaments and the localization of Myo5, the myosin motor head might contribute to translocate actin filaments away from the basal PM, and thereby push the endocytic coat into the cytosol (Fig. 1, stage 3).

Much less is known about the putative roles of actin previous and after elongation of the tubular endocytic profiles. Sun et al. demonstrated that the NPFs Las17 and Pan1 share an early function in endocytic uptake, previous to initiation of the slow inward movement [15]. The immuno-EM data indicates that initial membrane bending associated with coat assembly occurs previous to massive actin polymerization, but Las17 and Pan1 are already present on the surface of the endocytic coat at early stages. These NPFs could participate in the formation of an actin network that serves to subsequently anchor the actin filaments organized by Myo5 from the invagination base (Fig. 1, stages 1 and 2).

Actin and myosin might also be involved in vesicle fission, once the tubular profile has elongated (Fig. 1, stage 4). Genetic interactions between amphiphysin and type I myosin mutants [28] and the observation that some type I myosin mutants accumulate long PM invaginations [26] suggest that these proteins cooperate in a late budding stage. In mammalian cells, scission of clathrin-coated pits requires the GTPase dynamin, which might work as a regulatory enzyme and/or as a molecular motor that constricts the vesicle neck [30]. Strikingly, dynamin does not play a major role in endocytic uptake in yeast [31,32] and the role of actin in fission could not be investigated since Latrunculin A treatment blocks the process at an earlier stage [21]. Interestingly, the EM studies indicate that in the longest tubular profiles, actin and Myo5 rearrange to form two distinct pools associated with the invagination base and tip, leaving an intermediate region occupied by amphiphysin [28]. The acto-myosin structures might generate tension along the endocytic profile to cause fission in concert with a possible tubulation activity of Rvs161 and Rvs167 [33,34] (Fig. 1, stage 4). Further ultrastructural analysis of the primary endocytic profiles in different mutants will be required to dissect the actual dynamin-independent and possibly actin and type I myosin-dependent fission mechanism.

In mammalian cells, a mandatory role for actin in clathrin-mediated endocytosis has been controversial. However, TIRF (total internal reflection fluorescence) microscopy has clearly demonstrated that actin polymerizes on at least a subset of clathrin-coated pits assembled at the PM [35]. Increasing evidence also indicates that the machinery controlling actin dynamics at the sites of endocytosis is quite conserved (Fig. 2) and therefore, some actin and type I myosin roles in endocytic budding might be preserved throughout evolution.

Similar to yeast, the Arp2/3 complex, N-WASP (neuronal-WASP) and the unconventional type I myosin MyoIE are recruited to clathrin-coated pits assembled at the PM [35,36]. Further, interfering with their function can prevent clathrin-mediated endocytosis [36–38]. Dynamin directly binds to MyoIE and indirectly to N-WASP via multiple intermediates [39–45]. It is tempting to speculate that, comparable to yeast, different bursts of actin polymerization occur during budding, which could be coordinated via sequential association of dynamin with different partners. Consistent with this view, Yarar and co-workers demonstrated using EM, that Latrunculin A treatment has effects on multiple steps during clathrin-dependent budding in mammals [46]. However, the molecular functions of Arp2/3-dependent actin polymerization and type I myosin in mammalian endocytic budding are far from being understood.

Even though a conserved role of actin and type I myosin in membrane invagination and/or vesicle scission from the PM seems likely, it is still puzzling why actin is required for only a subset of clathrin-dependent endocytic budding events in mammals whereas it is mandatory for clathrin-mediated inter-

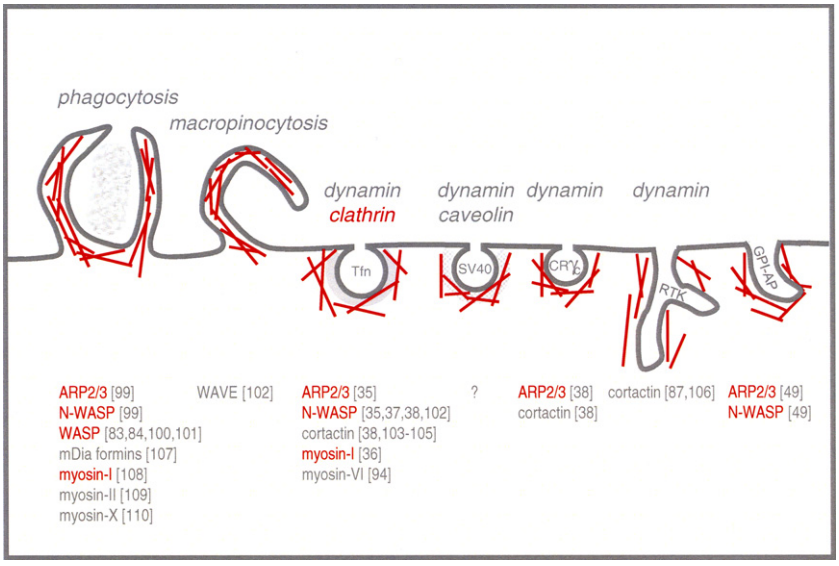

Fig. 2. Proteins involved in the control of actin dynamics at the sites of endocytic uptake in mammals. Besides the well-established role of actin in phagocytosis of solid particles [83,84,99] and in macropinocytosis [60], actin is required for numerous endocytic pathways, defined according to the morphology of the primary endocytic profiles, the proteins involved in their formation and/or the nature of the internalized cargo [47]. Actin polymerization facilitates clathrin and dynamin-dependent endocytosis at the apical membrane of epithelial cells [54] and polymerizes on the surface of at least a subset of clathrin-coated pits assembled at the PM (Tfn) [85]. Actin is also required for the dynamin-dependent internalization of SV40 via *caveolae* [86], for the dynamin-dependent but caveolae and clathrin-independent internalization of cytokine receptor γ chain (CRγc) [38], for the dynamin-dependent internalization of receptor tyrosine kinases associated with circular dorsal ruffles (RTK) [87] and for the dynamin, clathrin and caveolae-independent internalization of GPI-anchored proteins (GPI-AP) [49]. Arp2/3 seems to be a key player in most, if not all, internalization pathways. Distinct NPFs including WASP, N-WASP and WAVE activate the actin nucleating complex during different steps of the internalization process. In addition, cortactin, another NPF that stabilizes Arp2/3-dependent actin filament branching has been implicated in clathrin-mediated endocytosis and in the dynamin-dependent internalization of CRγc and RTKs. Besides actin polymerization nucleated by Arp2/3, the formins mDia1/2 can also promote actin polymerization on incipient phagosomes. Finally, the mechanochemical activity of several myosins play important roles in phagocytosis and budding of clathrin-coated pits. References supporting the role of the different proteins in the indicated pathways are given. Proteins labeled in red have counterparts in yeast that also function in endocytic uptake. Cortactin does not have a bona fide homologue in yeast but its domain structure and biochemical activities are similar to the yeast Abp1 [88,100–110].

nalization in yeast. Numerous results indicate that a number of clathrin-independent endocytic pathways associated to cholesterol-enriched lipid rafts require actin polymerization and share at least part of the machinery that controls actin polymerization on clathrin coated pits (Fig. 2) (reviewed in [47,48] and [38,49,50]). Interestingly, the yeast PM also has a high sterol content compared to mammalian cells [51,52]. Sterols increase the thickness and the rigidity of the lipid bilayer [53]. Thus, dependent on the sterol concentration of the incipient bud, clathrin and/or dynamin might need the forces contributed by actin and myosin to deform the plasma membrane and to produce a vesicle. Consistent with this hypothesis, actin polymerization is not required for clathrin-dependent uptake from the basolateral PM of epithelial cells but it is mandatory on the cholesterol-rich apical surface [53,54]. It will be interesting in the future to investigate to what extent clathrin and dynamin-dependent and -independent pathways share the molecular roles of actin and myosin in membrane deformation and vesicle fission.

2. Actin dynamics on intracellular endocytic profiles

Previously unnoticed, increasing evidence indicates that actin is associated with endosomes in yeast and it might facilitate post-internalization traffic. Besides a clear role of actin in vesicle and endosome motility, numerous results also point to its direct involvement in membrane fusion. In mammals, even though long-range endosome motility is clearly associated with microtubules, actin also contributes to endocytic traffic. Increasing evidence now suggests that some of the molecular roles of actin along the endocytic pathway could be evolutionary conserved (Fig. 3).

In *S. cerevisiae*, the primary endocytic vesicles scissed from the PM travel into the cytosol at about 230 nm per second, initially following an undirected trajectory [21,55] (Fig. 3, stage 1). The molecular mechanism involved in this trafficking event is at the moment unclear. However, it might involve Arp2/3-dependent rocketing since the complex still associates with the moving vesicles after the endocytic coat disassembles [21]. Consistent with this hypothesis, depletion of the yeast capping protein (Cap1/2), a protein necessary for reconstitution of bacteria actin-based rocketing in vitro [56], partially prevents the fast vesicle inward movement [57]. Intriguingly though, Pan1, Las17 and the type I myosins do seem not to travel with the vesicles into the cytosol [15,21,26] and depletion of Abp1, the only NPF still associated with them, does not prevent vesicle motility [20]. These observations indicate that either another NPF is in charge of activating the complex on the moving profiles or, once actin polymerization has been initiated, the Arp2/3 complex is only required to support the branched structure of the actin tail [6].

After the initial undirected traffic, a fraction of the primary yeast endocytic vesicles move away from the plasma membrane in association with actin cables, at a velocity close to 390 nm per second [22,55] (Fig. 3, stage 2). The yeast actin

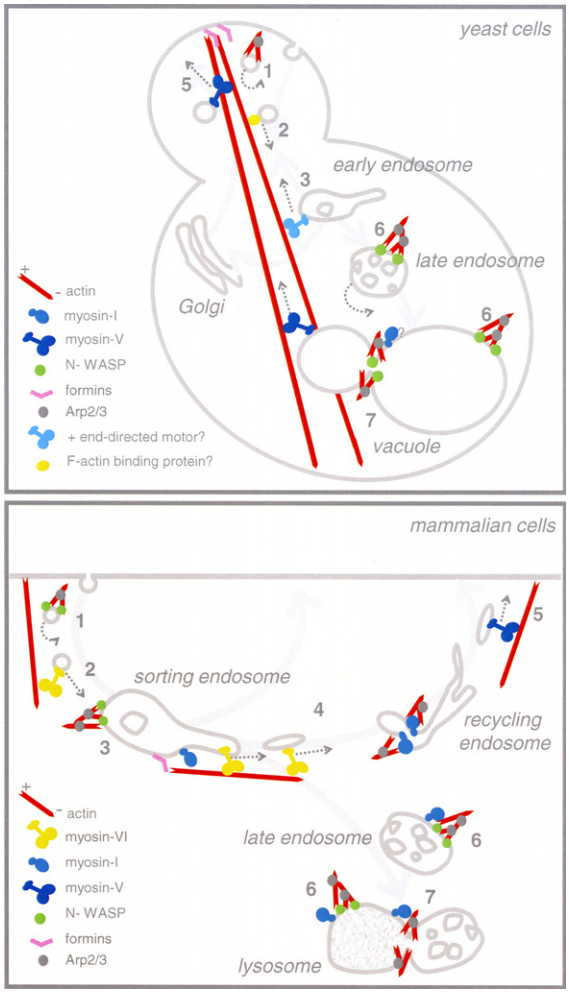

Fig. 3. Comparative roles of actin and myosin along the endocytic pathway in mammalian cells and yeast. (1) Upon completion of endocytic budding from the PM, vesicles might travel into the cytosol at the tip of actin tails, both in mammalian cells and yeast [21,57,60,61]. (2) In yeast, vesicles move toward the mother cell attached to actin cables induced by formins at the bud tip [22,55]. In mammals, subsequent directed movement of uncoated vesicles along actin cables involves the function of the minus end directed motor myosin-VI [62]. (3) Early endosomes in yeast move towards the endocytic vesicle before they fuse [22]. In mammals, endosome move along microtubules. However, formins and Arp2/3-dependent actin polymerization might contribute to modulate its directionality and speed [61,64,66,89]. (4) In addition, actin filaments nucleated on endosomal membranes might be required to support the role of type I and VI myosins in transport from early sorting to recycling endosomes [90–93]. Similar to the situation at the PM [36,94], type I and type VI myosins might cooperate in budding from early sorting endosomes. Myosin-VI could also carry transport intermediates away from the early sorting endosome. In addition, an intriguing role for the type I myosin myr4 in fusion between early sorting and recycling endosomes has been proposed [93]. (5) Myosin-V in mammals plays a role in transport from the early recycling endosome to the PM (reviewed in [63]). Type V myosin in yeast could also carry Golgi or endosome derived vesicles containing endocytosed cargo recycled to the PM [95,96]. (6) In yeast, late endosomes and vacuoles nucleate actin polymerization that can contribute to organelle motility [73,79]. In mammalian cells, actin and type I myosin play a role in traffic from late endosomes to lysosomes [72,77,97] and they might contribute to modulate endosome or lysosome motility [97,98]. (7) In addition, a direct role of actin in homotypic vacuole fusion has been demonstrated in yeast [75]. In mammalian cells, a role for actin in fusion of late endosomal and lysosomal compartments has also been proposed [78].

cables are bundles of highly dynamic actin filaments whose polymerization is induced by formins localized at the tip of the growing bud [58]. Formins are proteins that nucleate and support elongation of unbranched actin filaments in response to activation by Rho GTPases [7]. The actin cables are used for polarized transport of secretory vesicles, mRNA and organelles from mother to daughter cells [58]. Most transport events along actin cables depend on the type V myosins Myo2 and Myo4, which move toward the actin filament plus ends situated at the bud tip [58]. In contrast to secretory vesicles and organelles, endocytic vesicles move backwards, toward the mother cell [22,55] (Fig. 3, stage 2). This type of movement would require a minus end directed molecular motor such as myosin-VI [59], which does not exist in yeast. Interestingly, it was demonstrated using a GFP construct that discontinuously labels actin cables, that endocytic vesicles do not move along the actin cables but rather with them, as they elongate by addition of actin monomers at the bud tip [55]. The molecular machinery attaching the vesicles to actin cables has not been identified yet (Fig. 3, stage 2).

Directed backward movement of primary endocytic vesicles in yeast possibly facilitates their fusion with early endosomes [22]. Interestingly, it was shown that early endosomes move toward the primary endocytic vesicles along the actin cables before they merge [22] (Fig. 3, stage 3). Early endosomes can even engulf forming vesicles at the PM when endocytic uptake is delayed [22], thus providing a putative mechanism for fast recycling. Forward movement of early endosomes along actin cables does not seem to require any of the yeast type V myosins and the molecular motor possibly involved still needs to be identified [22] (Fig. 3, stage 3).

In mammals, an initial Arp2/3-dependent movement of nascent endocytic vesicles followed by a directed actin-dependent traffic away from the PM might be conserved (Fig. 3, stages 1 and 2). Nascent pinosomes moving at the tip of actin tails has been reported on macrophages using live-cell microscopy [60]. Pinosomes seem to recruit the actin polymerization machinery as they are being formed at the PM and they are subsequently propelled into the cytosol with a velocity similar to bacteria rocketing at the tip of actin tails [60]. Also, transferrin (Tfn)-labeled membrane profiles, possibly corresponding to primary clathrin-uncoated endocytic vesicles, have been observed moving at the tip of actin tails that co-localize with the Arp2/3 complex in vivo [61] (Fig. 3, stage 1). In addition, Tfn-labeled profiles travel toward the cell center in a myosin-VI-dependent manner [62] (Fig. 3, stage 2). Similar to the movement of endocytic vesicles associated with actin cables in yeast, Myosin-VI transports vesicles away from the actin filament plus end and thus, away from the surface where actin filaments are nucleated [59] (Fig. 3, stage 2).

In contrast to yeast cells, long-range directed movement of mammalian early endosomes occurs in a microtubule-dependent manner (for review see [63]). Nevertheless, mammalian early endosomes can nucleate Arp2/3-dependent actin polymerization under some experimental conditions (Fig. 3, stage 3) [64–67] and Rho-induced recruitment of formins on these organelles modulate their motility in vivo [68–71]. Even though the significance of actin polymerization per se on early endosomes is not well understood, numerous results indicate the essential role of actin and myosin along the endocytic recycling pathway in mammals [72] (Fig. 3, stages 4 and 5 and references therein).

Similar to nascent endocytic vesicles and endosomes, yeast late endocytic compartments also move in an actin-dependent manner (Fig. 3, stage 6). Chang et al. used a GFP-tagged version of the G-protein coupled receptor Ste2 to label intracellular compartments [73], which were later shown to most likely correspond to late endosomes [22]. The Ste2-GFP compartments move in an undirected manner sometimes making tight turns at a speed of 150–190 nm per second [73]. In contrast to the directed movement of early endosomes, disruption of the yeast cables does not affect the nature of the movement or the speed of the Ste2-GFP-labeled organelles, whereas Latrunculin A treatment or mutation of WASP/Las17, significantly slow them down. Strikingly also, fusion of the Las17 domains required for Arp2/3 activation to the Ste2 cytoplasmic tail recovers organelle motility in a *las17* mutant [74]. The authors proposed an actin rocketing-based mechanism to explain the movement of Ste2-GFP labeled endosomes. However, actin tails associated to these compartments have not been demonstrated and depletion of the yeast capping protein does not seem to affect the process [57].

The capacity of late endosomes to nucleate Arp2/3-dependent actin polymerization might also be conserved in higher eukaryotes. Late endosomes and lysosomes from Hela cells are capable of nucleating actin tails upon activation of protein kinase C on *Xenopus* extracts [67]. However, the physiological significance of the Arp2/3-driven late endosomal motility in vivo has not been investigated in detail yet (Fig. 3, stage 6).

Besides the well-established role of actin in membrane budding from the PM and vesicle and organelle motility, increasing evidence demonstrates that actin also has a direct role in fusion of yeast vacuoles (Fig. 3, stage 7). Eitzen et al. pointed out that yeast bearing mutations in Arp2/3, Las17, Vrp1 (The yeast WIP, wasp interacting protein) and the type I myosins have fragmented vacuoles, a feature of strains defective in homotypic fusion of the yeast lysosomal compartment [75,76]. Arp2/3, Las17 and Vrp1 are enriched in vacuoles and interfering with their function prevents vacuolar fusion in vitro [75]. Working under experimental conditions that discriminate between vacuole priming, docking and fusion reactions, the authors demonstrated that actin depolymerization is first required to allow vacuole docking, whereas actin polymerization is needed for the terminal steps leading to membrane fusion [75]. Actin accumulates at the vertex of docked vacuoles where fusion is thought to occur, suggesting a direct contribution of actin to distortion of the lipid bilayer [75].

Recent results indicate that actin could also be involved in fusion of late endocytic compartments in mammalian cells (Fig. 3, stage 7). Actin is required to transfer internalized cargo from late endosomes to lysosomes in vivo [72,77] and for in vitro fusion of late endosomes and lysosomes [78]. In addition, similar to yeast vacuoles, the late endosomal/lysosomal fraction is capable of inducing actin polymerization in this assay conditions [78,79]. The machinery involved in fusion of late endosomes with lysosomes in mammalian cells seems to very much resemble the biochemical requirement for homotypic vacuolar fusion in yeast [80]. Interestingly, it was recently shown that overexpression of one component of the HOPS complex, which also participates in docking of yeast vacuoles, induces clustering of late endosomes and lysosomes and promotes recruitment of actin, and type I and V myosins in NRK cells [81]. Investigation of the possible molecular roles of actin and myosin in membrane fusion in mammalian cells will require detailed analysis of the in vitro fusion reaction.

3. Perspectives

Analysis of endocytic uptake in yeast has very much contributed to our understanding of the molecular roles of actin and type I myosins in endocytic budding. The level of knowledge that allows building molecular models to explain endocytic membrane budding at the PM has required the effort of yeast genetics to identify the machinery involved, the development of live-cell imaging techniques and the ultrastructural analysis of the primary endocytic profiles. The reconstitution of the process using purified components will now be required to definitively nail down the forces contributed by the actin machinery in membrane bending or vesicle fission.

The role of actin in post-internalization endocytic traffic is only now starting to emerge. In most cases, we still need to define the functional significance of actin dynamics on endosomes and its exact contribution to cargo transport along the pathway. Molecular markers to unequivocally define the endosomal compartments in yeast and development of assays to quantitatively measure transit of cargo through those compartments in vivo would help detailing the exact roles of actin and myosin in post-internalization endocytic traffic. Even though the endocytic pathway is clearly more complex and subject to more numerous signaling inputs in mammalian cells, genetic analysis of the yeast post-internalization endocytic traffic might help unveiling some of the molecular roles of actin and myosin in endosome and vesicle motility and/or membrane budding and fusion.

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